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Clinical picture, epidemiology and outcome of *Loa*-associated serious adverse events related to mass ivermectin treatment of onchocerciasis in Cameroon

Michel Boussinesq*[1], Jacques Gardon[2], Nathalie Gardon-Wendel[2] and Jean-Philippe Chippaux[3]

Address: [1]Institut de Recherche pour le Développement (IRD), DSS, 213 rue La Fayette, 75480 Paris Cedex 10, France, [2]34160 Garrigues, France and [3]IRD, BP 1386, Dakar, Sénégal

Email: Michel Boussinesq* - boussinesq@ird.fr; Jacques Gardon - jacques.gardon@wanadoo.fr; Nathalie Gardon-Wendel - ; Jean-Philippe Chippaux - Jean-Philippe.Chippaux@ird.sn

* Corresponding author

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Abstract

In August 2002, 65 cases of *Loa*-associated neurological Serious Adverse Events were reported after ivermectin treatment. The first signs, occurring within the 12–24 hours following treatment, included fatigue, generalized arthralgia, and sometimes agitation, mutism, and incontinence. Disorders of consciousness, including coma, generally appeared between 24 and 72 hours, and showed a rapid variation with time. The most frequent objective neurological signs were extrapyramidal. The patients presented with haemorrhages of the conjunctiva and of the retina. Biological examinations showed a massive *Loa* microfilaruria, the passage of *Loa* microfilariae into the cerebrospinal fluid, haematuria, and an increase in the C-reactive protein, all of which have been correlated with the high intensity of the initial *Loa* microfilaraemia. Eosinophil counts decreased dramatically within the first 24 hours, and then rose again rapidly. Electroencephalograms suggested the existence of a diffuse pathological process within the first weeks; the abnormalities disappearing after 3–6 months. Death may occur when patients are not properly managed, i.e. in the absence of good nursing. However, some patients who recovered showed sequelae such as aphasia, episodic amnesia, or extrapyramidal signs. The main risk factor for these encephalopathies is the intensity of the initial *Loa* microfilaraemia. The disorders of consciousness may occur when there are >50,000 *Loa* microfilariae per ml. The possible roles of co-factors, such as *Loa* strains, genetic predisposition of individuals, co-infestations with other parasites, or alcohol consumption, seem to be minor but they should be considered. The mechanisms of the post-ivermectin *Loa*-related encephalopathies should be investigated to improve the management of patients developing the condition.

Definitions

Serious Adverse Events (SAEs), are defined as "ones which could be life-threatening, could result in permanent disability, or require hospitalization" (application text of the Mectizan® Donation Program, MDP). In the present paper, we shall address only the issue of those SAEs which occur after a standard dose of ivermectin (Mectizan®) for the treatment of onchocerciasis (150 μg/kg), and which are related to co-infection with *Loa loa*. Thus, we shall not consider the ivermectin toxicosis related to overdose and

passage of ivermectin through the blood-brain barrier [1], nor the uncommon cases of serious reactions, such as severe symptomatic postural hypotension or asthma attacks, reported from areas where loiasis is not endemic [2,3].

A first classification of SAEs was elaborated at a consultation organized by the MDP in Paris in October 1995. Briefly, the cases to be considered have (a) to occur in individuals who were previously healthy, (b) to be temporally related to treatment with Mectizan®, i.e. with central nervous system (CNS) signs and symptoms appearing within five days of treatment, and (c) to progress to coma without remission. In addition, the coma is usually associated with fever, and not with seizures. That being said, the cases were classified as (a) definite cases of *Loa* encephalopathy, (b) probable cases of *Loa* encephalopathy, and (c) coma events in a *Loa loa* endemic area. The definite cases were defined by the observation, in brain tissue, of microscopic findings consistent with *Loa* encephalopathy, i.e. a vasculopathy with evidence of *L. loa* microfilariae (mf) as a likely aetiology. The probable cases were defined by the presence of a *Loa* microfilaraemia above a given value (> 10,000 mf/ml if measured before treatment, or > 1,000 mf/ml if the sample is obtained after treatment) and/or the presence of *L. loa* mf in the cerebrospinal fluid (CSF). The last category includes cases of coma occurring in patients living in areas endemic for loiasis, who have been treated with ivermectin but for whom no laboratory results are available to support the diagnosis of *Loa* encephalopathy. An additional category of "possible cases of *Loa* encephalopathy" was added recently to include those patients for whom the *Loa* microfilaraemia was not assessed quantitatively, but who showed *Loa* mf in their blood smear.

Besides this classification, which is very useful, observations made during a study performed in the Lekie area of the Republic of Cameroon to evaluate the incidence rate of, and the risk factors for, post-ivermectin encephalopathies have led us to elaborate another complementary classification, aimed at describing the seriousness of the condition on the basis of the severity and duration of the functional impairment [4,5]. Four degrees have been defined.

1) Mild reactions; 2) Marked reactions; 3) Serious non-neurological reactions; 4) Serious neurological reactions.

1) Mild reactions are the ones that are not accompanied by any functional impairment.

2) Marked reactions are similar in nature to the mild reactions (headache, joint pains, itching, oedema) but their intensity is such that they are accompanied by functional impairment requiring, for several days, assistance in performing some everyday natural functions and household activities.

3) Serious non-neurological reactions correspond to conditions associated with functional impairment that require, for at last one week, full-time assistance; patients usually stay in bed or in armchairs and have difficulty in standing up or walking, but do not show disorders of consciousness or objective neurological signs.

4) Serious neurological reactions are associated with disorders of consciousness and neurological signs.

This classification has the advantage of being relatively simple to apply in the field, and of taking into account both the degree of impairment, and its duration. However, although we are aware that this may overwork the medical personnel (even the Glasgow coma score is difficult to obtain in medical records of the cases) when the patients are hospitalized, it could be useful to use more accurate scales, such as the Karnofsky performance scale or, may be better still, the palliative performance scale [6].

It is clear that the only life-threatening events are the serious neurological reactions. However, the other serious and marked reactions, which are much more frequent, have an important impact on the populations' perception of ivermectin, and play a major role in lowering their acceptance of the drug, as is often reported from those areas where loiasis is co-endemic with onchocerciasis.

Description of sources regarding clinical and biological features

From the inception of the Mectizan® Donation Program in 1988 to January 2002, the total number of SAEs reported to the MDP was 187, including 159 from Cameroon, 14 from Sudan, 6 from Nigeria, 5 from the Democratic Republic of the Congo (DRC), and one from the Central African Republic (CAR). In August 2002, the number rose to 207 (176 from Cameroon), out of which 65 were classified as probable or possible cases of *Loa* encephalopathy temporally related to treatment with Mectizan®. The present paper will summarize observations made on only 35 cases, all from Cameroon, for which we have some information. They include the five cases described in detail by Boussinesq *et al.*, [5], eight cases hospitalized in May 1999 at the Central Hospital of Yaoundé (CHY), 16 patients who also developed SAEs in May 1999, but for whom we have only the standardized form for reporting of SAEs, and 6 cases from the District of Malantouen (West Province).

Regarding the biological aspects, along with the data presented by Boussinesq *et al.*, [5], we have re-analysed some

results of the study reported by Ducorps *et al.*, [7]. This study, which aimed at evaluating the clinical and biological changes after ivermectin treatment in patients with a significant *Loa* microfilaraemia, was performed at the CHY. A total of 112 patients, each with more than 3,000 *Loa* mf per ml of blood, agreed to participate. These patients were resident in the Lekie area (Central Province, Cameroon). Twenty-six of them were co-infected by *Mansonella perstans*, (median load of 250 *M. perstans* mf/ml). No skin snips were taken before inclusion in the study, but these patients had been examined for onchocerciasis several months previously (maximum: one year). At that time, 52 of the 112 patients were co-infected with both *Loa loa* and *Onchocerca volvulus*, but the microfilarial counts of the latter parasite were very low: only 13 patients had 10 mf per skin snip or more, and the median *O. volvulus* microfilarial load in the infected individuals was 3 mf per skin snip.

Clinical picture

Clinical signs and symptoms within the 3 days following treatment

The vast majority of patients developing an SAE complained of various symptoms developing within the 12–24 hours following treatment (Day 1, D1). The main complaints were fatigue, which could be severe and accompanied by anorexia, headache, and generalized arthralgia. Among the latter, the patients often complained of severe lumbar pain, which could be objectively appreciated by the characteristic bent-forward walk of the patients. The subjects often said that they were "broken". Stomach pain and diarrhoea have also been reported at D1.

Besides these signs, which are classically reported after ivermectin treatment of patients not infected with *Loa loa*, but at a lower degree of seriousness, more alarming signs sometimes developed as soon as D1. These were confusion, agitation, dysarthria, mutism, and incontinence. However, more often these signs were reported later, at Day 2 (D2) or Day 3 (D3). In their study, Ducorps *et al.*, [7] measured the Karnofsky performance scale every day during the week after treatment. Among those patients with a pre-treatment *Loa* microfilaraemia exceeding 30,000 mf/ml, the proportions of patients presenting a functional impairment at D1, D2, D3 and Day 4 (D4), were 4%, 36%, 32%, and 37%, respectively. Thus, it appears that the neurological involvement generally appears first at D2. Although some cases of coma have been reported as soon as D2, this condition usually develops at D3 or D4.

It seems that the various signs related to oral expression (dysarthria, mutism, and confusion) are typical of the condition, as well as incontinence, which usually occurs in the course of development of the symptoms. This incontinence is often only urinary, but faecal incontinence may appear when the condition worsens.

All these signs are usually accompanied by fever, which tends to reach its highest level at D1, but is often reported only at D2–D3. The temperature may rise up to 40°C.

The neurological condition

Detailed information on the objective neurological signs shown by the patients has been presented by Boussinesq *et al.*, [5]. Very few additional data are available from the medical records and forms from the other cases, even those hospitalized at CHY in 1999. When the cases are appropriately managed, it seems that the worst Glasgow scores are recorded on D4–D5. However, when the patients develop complications (see below), the neurological condition may continue to worsen progressively. A striking feature observed in some patients is the rapid variation with time of the degree of disorders of consciousness. Generally, the patients do not have seizures. The only cases reported so far are Bi. G., a little girl aged 6 years, who had seizures at D13 and D19, without fever; and Ob. JJ, a male 20-years old, whose condition at D2 included generalized itching, followed by disorders of consciousness and later by seizures and vomiting. On systematic examination, motor or sensory deficits were not found, but tendon reflexes were most often abolished or diminished, though they were brisk in some cases. Usually, there was no Babinski's sign, but meningeal signs were found in some patients. The signs most constantly found were extrapyramidal. Hypertonia was frequent, and the cogwheel phenomenon was one of the last signs to disappear.

To summarize, the condition is very variable between cases and, for a given case, may change very rapidly from one examination to another. This variability over time seems to be characteristic of the condition.

Associated clinical signs and symptoms

Ophthalmology

One of the signs associated with the post-ivermectin *Loa*-related SAEs is the appearance of haemorrhages in the palpebral conjunctiva (HPC) and of retinal lesions (RL), described by Fobi *et al.*, [8]. The HPCs were rarely seen at D1, but were always present between D2 and D5. After D6, they tended to diminish, and they disappear without sequelae around 10 days after treatment. These HPCs have a typical appearance: the main trunk of the vessels appear to be normal, but a blot haemorrhage is present at the tip of the vessel (see photographs in [8]). Sometimes, several haemorrhages coalesce to form a single large haemorrhage. These lesions are located below the lid margin, whereas no lesions have been seen in the bulbar conjunctiva. A conjunctival biopsy collected at D5 from one

patient with a pre-treatment load of 86,400 *Loa* mf/ml showed marked vascular congestion, with erythrodiapedesis and interstitial haemorrhages; the vascular lumina were widely open and filled with red blood cells, but no mf were seen. This appearance suggested that the HPCs were due to a passive congestion caused by obstruction of the small vessels located downstream. The risk of developing HPCs after ivermectin treatment was found to be significantly increased when the *Loa* microfilaraemia exceeded 1,000 mf per ml (reference patients: those with 0 mf/ml). Thus, some patients may present HPCs without developing SAEs. However, due to their early appearance (D2), these lesions can be considered as a warning signal indicating patients who might be about to develop neurological reactions. In addition, it has been proposed to examine systematically for HPCs all patients exhibiting SAEs [8]: the presence of such lesions constitutes an argument in favour of the implication of *Loa* in the condition. Lastly, it might be useful to investigate the possibility that HPCs may occur spontaneously; such a phenomenon would probably be even less frequent than after treatment, but one may consider the possibility that this sign may help to identify patients with considerable *Loa* microfilaraemias.

Another feature reported from patients with post-ivermectin *Loa*-related SAEs is the existence of retinal haemorrhages and "cotton wool exudates" corresponding to areas of nerve fibre layer ischaemia. These are mainly located at the end of blood vessels, particularly temporal to the macula. The haemorrhages are usually intraretinal but can also be pre-retinal. They are mostly located at the tip of blood vessels, and often have one or two white spots in their centre, giving them an appearance close to that of Roth's spots (see photographs in [8]). In some cases tiny venules and arterioles appear completely empty, and have the aspect of white filaments. The interval between ivermectin treatment and appearance of these lesions is not accurately known, however, it seems that they are usually seen after D3, and that they may still be visible up to 3–4 weeks later. Such retinal lesions have also been seen in post-diethylcarbamazine *Loa*-related SAEs, and it appears that rare cases have also been reported in untreated patients infected with *Loa loa* (see review in [8]). As for the HPCs, we think that retinal lesions should be looked for when a patient develops a neurological condition possibly related to *Loa* infection, and that the presence of such typical lesions is a strong argument in favour of the implication of *Loa* in the condition.

Nephrology

Loiasis may be associated with proteinuria and/or haematuria, and it may provoke membranoproliferative and membranous glomerulonephritis [9–15]. However, several observations also show that ivermectin may provoke renal complications; and it has been shown (see below), that the drug induces the passage of *Loa* mf into the urine, and also haematuria. Sometimes, this can be associated with serious signs and symptoms. For instance, one patient described by Cruel *et al.*, [16] developed an acute nephropathy several hours after ivermectin treatment although he was harbouring only a fairly low *Loa* microfilaraemia (400 mf/ml). Examination of a renal biopsy in this patient showed numerous mf in the glomeruli, and the presence of immune complexes. The only patient who developed a coma in the study by Ducorps *et al.*, [7] also presented with glomerulonephritis, with a proteinuria at D3, and anuria at D4–D5. It is interesting to note that renal changes have been also reported after ivermectin treatment of humans with onchocerciasis [17], and after ivermectin treatment of *Dirofilaria immitis*-infected dogs [18].

Complications

It seems that the main complications of post-ivermectin *Loa*-related SAEs are related to a prolonged hospitalization, or to a long stay in bed. After 10–20 days, the comatose patients readily develop bedsores, and this is a signal of poor prognosis. Furthermore, the patients are at risk of dehydration, of deterioration of an underlying condition (e.g. diabetes), and of developing nosocomial (especially broncho-pulmonary) infections. Intense nursing is thus the main element of the management of the patients. But besides these non-specific complications, several cases of neurological sequelae, which are probably related to the pathological processes associated with the *Loa* encephalopathy, have also been reported (see under "Outcome" below).

Biological, and other complementary examinations

Parasitological examinations

Loa microfilaraemia decreases very sharply after ivermectin treatment: in the patients who participated in the study by Ducorps *et al.*, [7], and who had been treated with a single dose of 200 μg/kg, the loads recorded 24, 48 and 72 hours after treatment were about 40%, 25%, and 20%, respectively, of their initial values. The reduction rate is similar whatever the pre-treatment microfilaraemia. After D3, the loads continue to decrease, but in a very slow manner [19].

Ivermectin also provokes a massive *Loa* microfilaruria, whose incidence is correlated with microfilarial load. During the study reported by Ducorps *et al.*, [7], a *Loa* microfilaruria was rarely found before treatment, being observed in only 4% of those patients with a microfilaraemia above 30,000 mf/ml. At D1, *Loa* mf were found in the urine of 24% and 57% of those patients with initial loads of 15,000 – 30,000 and > 30,000 mf/ml, respectively. The

values recorded in these two groups were still higher at D3 (54 and 79%, respectively), and then decreased at D4. The microfilarial counts in the urine followed the same pattern over time, as did the prevalence, with a sharp decrease after D4. In all cases, these mf were mobile, and thus alive.

The most characteristic feature of the post-ivermectin *Loa*-related SAEs is the passage of *Loa* mf into the CSF. In the study reported by Ducorps *et al.*, [7] *Loa* mf were found at D1 in the CSF of 17%, 25%, and 13% of the patients harbouring pre-treatment microfilaraemias < 15,000, 15,000 – 30,000, and > 30,000 mf/ml, respectively. The corresponding figures at D3 were 25%, 60%, and 77%. To verify whether these mf were or were not in the CSF before treatment, lumbar punctures were done before treatment in 10 patients with *Loa* loads above 10,000 mf/ml, none of them had mf in the CSF before treatment, but four showed a microfilarorachia at D3. As in the urine, these mf were alive. It is difficult to assess at what interval after treatment the *Loa* mf disappear from the CSF. They have been found two weeks after, but the examinations done in the patient of Ducorps *et al.*, [7], who showed counts of 102 and 14 mf/ml of CSF at D6 and D13, respectively, suggest that the densities of *Loa* mf in the CSF decrease rapidly after one week.

These observations suggest that ivermectin treatment induces the *Loa* mf to flee from the blood circulation. Such a phenomenon had been reported in patients infected with *O. volvulus*, and treated with diethylcarbamazine (DEC) [20,21].

Proteinuria and haematuria

Before treatment, less than 10% of the patients included in the study by Ducorps *et al.*, [7] showed evidence of microscopic haematuria, whatever their microfilaraemia. This proportion increased progressively after treatment and, by D3–D4, 18% of patients with <15,000 mf/ml, 19% of those with 15,000–30,000 mf/ml, and 35% of the individuals harbouring >30,000 mf/ml developed haematuria. Ivermectin treatment also provoked the appearance of proteinuria, with an incidence of 70% in those patients with > 30,000 mf/ml [7].

Biochemical and cytological results in the CSF

Besides the mf (see above), and a moderate increase in the levels of proteins, which is observed in some cases only, no abnormalities have been recorded in the CSF.

Leukocytes, and in particular, eosinophils

The data collected as part of the study published by Ducorps *et al.*, [7] show an increase in the leukocyte counts between D0 (pre-treatment) and D3. The counts varied from 7,120 to 7,280 per mm^3 in the group with less than 15,000 mf/ml, from 7,510 to 8,080 per mm^3 in the patients with 15,000–30,000 mf/ml, and from 6,960 to 8,100 per mm^3 in the individuals harbouring more than 30,000 mf/ml. The same study shows that the eosinophil counts decrease dramatically within the first 24 hours after treatment, and that this decrease becomes more marked as the initial microfilaraemia increases. The counts recorded at D1 in the patients harbouring < 15,000, 15,000 – 30,000 and > 30,000 mf/ml were 84%, 62%, and 50%, respectively, of the pre-treatment counts recorded in these 3 groups. At an individual level, there was a positive correlation between the drop in the microfilaraemia from D0 to D1, and the decrease in the eosinophil counts during the same interval. After this dramatic decrease, the eosinophilia rose again rapidly, and at D3, the values recorded were 120–130% of the initial counts in all the three groups of patients. The changes after D3 were not studied by Ducorps *et al.*, [7], but Martin-Prével *et al.*, [22] reported a marked increase in the numbers of eosinophils 8–10 days after treatment with ivermectin, and then a slow decrease in the counts, with values similar to, and less than half of, the pre-treatment ones, at D23–D33, and D92–D109, respectively.

As indicated above, 52 of the 112 patients included in this study were also co-infected with *O. volvulus*, but with very low microfilarial loads. We have compared, within each group of patients, defined according to their *Loa* microfilaraemia (< 15,000; 15,000 – 30,000; and > 30,000 *Loa* mf/ml), the mean decrease in the eosinophil counts in the *O. volvulus* snip positive and the *O. volvulus* snip negative patients. In the two groups with the highest *Loa* loads, the values were similar, but with a slightly greater decrease in the patients with no skin *O. volvulus* mf (in the patients with 15,000 – 30,000 *Loa* mf/ml: decrease of 633 vs. 698 eosinophils per mm^3; and in the patients with > 30,000 *Loa* mf/ml: decrease of 602 vs. 722 eosinophils per mm^3). In both groups, the mean *Loa* microfilarial loads were similar for snip positive and snip negative patients. Thus, it seems that, in this study, infection with *O. volvulus* played a minor role in the decrease in eosinophilia between D0 and D1, and that the phenomenon can be attributed to the infection with *Loa*.

Besides these global trends, one should note that in some patients who develop *Loa*-related post-ivermectin SAEs, a dramatic drop in the eosinophilia can be observed a number of days after treatment. This was the case in two patients reported by Boussinesq *et al.*, [5]: the patient described as "case 1" had a nil eosinophilia at D13; and in "case 3" the eosinophil counts at D4 and D7 were 1,700 and 0 per mm^3, respectively. This same phenomenon was also observed in two of the patients who were hospitalized at the CHY, in 1999, and who had a full blood count. One of them (Ol. Aw. L.) had 41 eosinophils per mm^3 at D6, and the other (On. Ay. S.) had a nil count at D11.

The global changes in eosinophil counts recorded after ivermectin treatment in patients infected with *L. loa* are similar to those recorded by Ackerman *et al.*, [23] and Cooper *et al.*, [24] in onchocerciasis patients after treatment with DEC or ivermectin. These authors arrived at the conclusion that the "Mazzotti reaction", and its severity, were associated with eosinophil sequestration and activation-degranulation. This feature confirmed previous observations on the preponderant role of eosinophils in the development of the Mazzotti reaction [25–27]. Similar processes probably play a major role in the initiation of the post-ivermectin *Loa*-related reactions, however, this may not account for all the signs making up the picture of post-filaricide *Loa*-related SAEs.

Other elements related to inflammation

No data are available concerning the changes in the erythrocyte sedimentation rate after ivermectin treatment of patients with *Loa loa*. In the study by Ducorps *et al.*, [7], a marked increase in the C reactive protein (CRP) was observed within the 3 days following ivermectin treatment. The concentrations increased from 5.0 to 12.0 mg/l in the group with initial loads < 15,000 mf/ml, from 1.0 to 24.1 mg/l in the group with 15,000–30,000 mf/ml, and from 5.0 to 53.3 mg/l in the group harbouring > 30,000 mf/ml. It has also been shown in another study group that the CRP levels decrease between D3 and D7 [19]. In the study by Ducorps *et al.*, [7], a slight but significant increase in the complement component 3 (C3) has been recorded between D0 and D3: the values changed from 0.71 to 0.74 mg/ml in the group with the lowest *Loa* counts, and from 0.65 to 0.69 mg/ml in the two other groups.

Other biological examinations

Ducorps *et al.*, [7] reported a moderate but significant decrease (by about 15%) in the alkaline phosphatase between D0 and D3, whereas the mean values of aspartate and alanine aminotransferases (ASAT and ALAT) remained stable. A moderate increase (by 10%) in the creatine phosphokinase (CPK) was also reported by these authors.

Electroencephalography

Repeated electroencephalograms (EEG) were done in two patients who participated in the trial by Gardon *et al.*, [4]. As indicated by Boussinesq *et al.*, [5], one patient showed, "at D15, periodic occurrence, during hyperventilation, of diffuse discharges of large amplitude; on D146, the tracing was asymmetric and characterized by slow activity with additional spikes, indicating focal activities in the right parieto-occipital area, which worsened during hyperventilation; on D233 the abnormalities had disappeared and the tracing did not show any pathologic activity". The EEG done on D19 on the other patient showed "a slow tracing with spontaneous occurrence of diffuse, paroxysmal, and monomorphic theta activity that lasted 2–3 sec.; the slowing of activity was increased by hyperventilation; by D105 an improvement was recorded but focal abnormalities persisted in the left occipital region, especially during hyperventilation; by D159 all previously recorded abnormalities had disappeared".

These results suggest the existence of a diffuse pathological process in the brain within the first weeks; that lesions are still present 3–6 months after the SAE; and that EEG abnormalities disappear only after this interval. It should be noticed that in the first of the two cases reported, the patient, in spite of his normal EEG after 7 months, may be considered as having mild sequelae. Although this may be regarded as a subjective observation, his relatives said that his mood had changed and that he was significantly calmer than before treatment.

Computerized axial tomography

Tomography was performed in two of the cases of *Loa*-related post-ivermectin SAEs hospitalized at the CHY in April 1999. In one case, the examination was done at D14, and showed a cortico-sub-cortical atrophy (probably related to pre-existing vascular phenomena), and hypodense areas of the brain stem (lateralised at the right side), and in the inner temporal areas (at both sides); there was no sign of cerebral oedema. In the other case, the examination was done 21 days after treatment, and no abnormalities were found.

Outcome

Recovery without sequelae

When appropriately managed, most of the patients developing *Loa*-related post-ivermectin SAEs recovered completely, without sequelae. The neurological examination became normal after several weeks, usually before one month, the last signs to disappear being the extrapyramidal signs (cogwheel phenomenon). As indicated above, the EEG still showed abnormalities after several months, but became normal within 6–7 months.

Death

If the patients remain confined to bed for a long time they may develop complications, the most frequent one being bedsores. As the latter are most often, in the African context, not appropriately managed, superinfections appear and the patient may die after several weeks from septic complications, dehydration, and malnutrition. Fairly detailed information is available for several patients who died from *Loa*-related post-ivermectin SAEs, and may be summarized as follows (all patients developed bedsores, which are not noted below): (i) case described in [28]: death at D25, due to gastrointestinal bleeding possibly due to corticosteroid treatment; (ii) case 1 of [5]: death at

D21, partly due to a large abscess on one cheek; (iii) Ol. Aw. L. (dead at CHY in 1999): death at D18 due to a septic shock probably favoured by a pre-existing diabetes; (iv) On. Ay. S. (dead at CHY in 1999): death at D48, from progressive decline; (v) Nt. D. (dead at CHY in 1999): death at D6, high fever, cause unknown; (vi) Tc. J. (dead at Malantouen hospital in 1999): death at D6, high fever, cause unknown.

Recovery with sequelae

In other cases, the patients recover progressively, but with more or less severe sequelae. The latter can be summarized as follows: (i) case 2 of [5], change in mood, according to the relatives, calmer than before treatment; (ii) case 3 of [5], episodic amnesia, leading to interruption of school attendance; (iii) Bi. G. (hospitalized at CHY in 1999), dysphasia; (iv) On. A. (hospitalized at CHY in 1999), "scatterbrained and less gallant" than before treatment, according to the family, cogwheel phenomenon; (v) No. JM (hospitalized at Okola Hospital in 1999), walking disorders, cogwheel phenomenon.

These sequelae are a cause of concern, but in all cases they are much less severe than those recorded in patients who developed SAEs after DEC treatment.

Epidemiology

Main risk factor: Loa loa *microfilaraemia*

Demonstration

The fact that the *Loa* microfilaraemia is by far the main risk factor for post-ivermectin SAEs has been demonstrated by Gardon *et al.*, [4]. This result is consistent with all the previous observations on the occurrence of SAEs after other filaricide treatment, especially DEC. It is probably true that co-factors also exist, because not all subjects with high *Loa* microfilaraemia develop an SAE after ivermectin, but we feel that they play a minor role, and that further research to prevent or manage the post-ivermectin SAEs should focus on the pathological processes involving *Loa loa* itself.

Thresholds of risk

The criteria for defining probable cases of *Loa*-related encephalopathy include threshold values for *Loa* microfilarial loads (>10,000 mf/ml if measured before ivermectin treatment, or >1,000 mf/ml if the sample is obtained after treatment). As these values were determined in 1996, and although determining such "threshold" values may be regarded as artificial because of the progressive aspect of the phenomenon, one may wonder whether they are still valid in 2002.

Concerning those SAEs with disorders of consciousness, information on the pre-treatment *Loa* microfilaraemia is available for only 5 cases [5,29]. In all of these, the counts were >50,000 *Loa* mf per ml. It is interesting to notice that this value is exactly the same as the one presented by Fain [30] as being the threshold above which there is a risk of post-DEC *Loa* encephalopathy.

Besides the cases with objective neurological signs, Gardon *et al.*, [4] have defined serious non-neurological cases, i.e. with no objective signs but a functional impairment requiring full-time assistance for at least one week. The incidence of such reactions is closely related to the *Loa* microfilaraemia and may be estimated using the following formula [4,31]:

$$\text{Probit (incidence)} = 3.96 + (1.87\ [\log_e(x) - 11.06]),$$

where x is the *Loa* microfilaraemia per ml.

When one replaces x by various values, one obtains the following results for the probability of developing a serious reaction (whether or not neurological) after ivermectin treatment: 0.7% when x = 30,000; 2.7% when x = 40,000; 7.0% when x = 50,000; 27.0% when x = 80,000; 42.0% when x = 100,000; 71.0% when x = 150,000; and 97.0% when x = 300,000 mf/ml.

From these results, one may consider that the value of 10,000 *Loa* mf/ml, which has been proposed as a criterion for defining "probable case of *Loa*-related encephalopathy" is fairly low. However, we would be inclined to keep this value for the time being, because the quantitative assessment of mf concentrations in blood smears by microscopy is often not very accurate.

Besides this, one should consider the values of microfilaraemia recorded after treatment, which are of course the only ones which are usually available. As indicated above, the *Loa* microfilarial loads recorded 24, 48 and 72 hours after treatment in patients who received ivermectin at a dose of 200 μg/kg were about 40%, 25%, and 20%, respectively, of the initial values. In the trial reported by Kamgno *et al.*, [19], the number of patients, as well as their *Loa* loads, were lower than those reported in the study by Ducorps *et al.*, [7], but the patients were treated with the standard dose of ivermectin recommended for treatment of onchocerciasis, and the follow-up was done every day from D0 to D7, and then on D15 and D30. From all these data, one may consider that after a single dose of 150 μg/kg, the *Loa* loads at D1–D3, D4–D7, and D7–D15 are about 25–40%, 20–25%, and 15–20%, respectively, of the initial values. This would mean that the threshold values for defining a probable case of *Loa*-related post-ivermectin SAEs would be 2,500–4,000, 2,000–2,500, and 1,500–2,000 mf/ml, respectively, if the blood smear has been done at D1–D3, D4–D7, or D7–D15 after a standard dose of ivermectin. However, one

should keep in mind that the response to treatment may vary widely between individuals, and that should the severity of the reactions be also related to the rapidity of the decrease in the microfilarial loads, lower values than those quoted above might be recorded in some patients after treatment. The values obtained at longer term (3, 6 or 12 months after a first single dose) have been presented by several authors [32–34]. The results show that, in those patients with more than 30,000 mf/ml, the individual risk of developing a SAE related to *Loa* infection would be reduced by over 90% if a second Mectizan® treatment were given 6–12 months after the first [34].

Other possible risk factors

Even though it is clear that high *Loa* microfilaraemia is the main factor associated with the risk of developing a post-ivermectin *Loa*-related SAE, one may wonder whether other factors may act as co-factors. Our studies show that not all the patients whose *Loa* loads exceed the threshold of risk develop an SAE. One may also wonder whether some, as yet unrevealed, specific factor exists in Cameroon, where most of the cases of SAEs have been reported.

Loa loa *parasitic strains*

Even if neurological SAEs have been reported from many countries such as Gabon [35], CAR [36], Democratic Republic of the Congo and Sudan, most (about 85%) of the post-ivermectin SAEs have been reported from Cameroon. This is probably mainly related to the fact that the *Loa* microfilarial loads are higher in the latter country, and/or to differences in the surveillance procedure during the distributions, but it might also suggest that the local parasitic "strain" in Cameroon is more pathogenic than the others. In this respect, parasite samples collected in Cameroon, Gabon, Nigeria, Congo were sent to Dr. T. Unnasch, who analysed the genetic polymorphism in potentially interesting loci from the *Loa loa* genome. These studies are still ongoing. However, it is clear that even if a specific parasite population exists in Cameroon, this does not necessarily mean that this specificity is associated with the risk of being involved in development of SAEs. In addition, one should keep in mind that the epidemiology of the post-ivermectin *Loa*-related SAEs is probably similar to that of the post-DEC encephalopathies, and that, regarding the latter, it is clear that there was no geographic clustering of the cases. Assuming this, one should consider that one of the main areas at risk, presently, is located in the DRC, where so many cases of post-DEC SAEs were seen (80–90 cases within 10 years in a single hospital in the Mayumbe area [30]) that the physician who managed them did not feel that it was useful to publish any description.

Genetic predisposition of some populations

Again, the high number of cases reported from Cameroon may suggest that the local human population has specific risk cofactors, possibly genetic ones. The arguments presented in the preceding paragraph also apply when considering this possibility.

Predisposition of some individuals

At an individual level, it has been demonstrated that sex is not a factor associated with an increased risk of developing a serious reaction to ivermectin. However, susceptibility does appear to be age-related [4]. For a given *Loa* load, people aged 30–44 develop SAEs significantly more often ($P = 0.032$) than those in the reference class (age 15–29). Conversely, the risk is not increased for persons aged 45–59, and those aged 60 and above. This result is difficult to explain, but it is possible that specific co-factors exist more often in individuals aged 30–40. Logistic regression analyses have also been done on the factors associated with the occurrence of haemorrhages of the palpebral conjunctiva, a feature which is part of the global picture of the SAEs. In this analysis, it was found that the risk of developing an HPC was significantly higher ($P < 0.001$) in males; conversely, age was not found to be associated with the occurrence of HPCs [8].

Co-infestations

Co-infestation with *O. volvulus* does not seem to have to be regarded as a cofactor associated with the SAEs. Many cases of SAE were reported from patients living in areas where onchocerciasis is hypoendemic or non endemic. This was observed in patients who came from the District of Okola (located some 20 km north-west of Yaoundé, in the Lekie Division), and who were reported in April 1999, and in two patients who developed disorders of consciousness in Ngat (village located some 50 km south of Yaoundé, in the Nyong and So'o Division), where loiasis is highly endemic, and where there is no transmission of onchocerciasis [29]. Two of the patients who developed an SAE had undergone both a blood smear and a skin biopsy before treatment. The first one is the one described in [28]; the skin snip was positive, but the microfilarial load is unknown. The second case is the one described by Ducorps *et al.*, [7]; no mf of *O. volvulus* was found in the skin of this patient.

In the analysis performed by Gardon *et al.*, [4], *M. perstans* microfilaraemia was not found to be significantly associated with the development of serious reactions, but the P-value (0.054) was near the limit of significance. In the study on the factors associated with the occurrence of HPCs, it was found that, besides the *Loa* microfilarial load, which was the main factor, and male sex, *M. perstans* microfilaraemia was also a variable associated with the risk of developing HPCs, with a P-value of 0.012 [8]. This

is probably explained by the existence in the human population of a positive association between *Loa* and *M. perstans* infections [37,38].

Njoo *et al.*, [39] performed a study on onchocerciasis patients treated with ivermectin, and did not find any relationship between the occurrence and extent of side effects and the severity of concurrent intestinal parasitic infections (*Ascaris lumbricoides*, *Trichuris trichiura*, hookworm, *Schistosoma mansoni*).

The classification of the cases produced in October 1995 specified that, besides the required criteria, a helpful piece of information to define probable cases of *Loa*-encephalopathy would be the absence of *Plasmodium* in the blood smear. This is indeed an important issue to address because the clinical pictures of *Loa*-related SAEs and cerebral malaria present many similarities, including fever, disorders of consciousness, and even retinal haemorrhages with white centres [40].

Examination for *Plasmodium falciparum* has been done in only some of the cases of post-ivermectin SAEs. In most cases, no malaria parasite was found, but in some cases high numbers of *Loa* microfilariae were associated with *Plasmodium*. Whether the presence of *Plasmodium* can facilitate the development of *Loa* encephalopathy is not known.

The areas of Cameroon where the cases of post-ivermectin SAEs come from are not endemic for trypanosomiasis. The clinical condition of the patients before treatment did not suggest that they could be infected with *Trypanosoma*. However, patients who presented both *Loa* mf and *Trypanosoma* in the CSF have been reported from Southern Sudan and DRC. Additional data are required to document the possible interactions between *Loa loa* and *Trypanosoma*, and to evaluate whether the co-infected patients would have to be treated by ivermectin.

Alcohol consumption

In a number of cases, the relatives of the patients who developed SAEs reported that the patient had ingested alcoholic beverages after ivermectin treatment. This is true for the patient described in [28], who had drunk palm wine and red wine just after having been treated.

Very few data have been published on the plasma concentrations of ivermectin after different doses of ivermectin [41]. But one may imagine that if alcohol increases the availability of ivermectin, then the concentration of drug in the blood would be higher, as would be the effect on the mf. The first indication that alcohol intake influences the bioavailability of ivermectin was reported by Edwards *et al.*, [42] who observed that the systemic availability of a 12-mg dose of ivermectin was approximately doubled when the dose was in the form of alcoholic solution, as compared with capsules or tablets. The only study on the effect of co-ingestion of ivermectin tablets and alcohol was performed by Shu *et al.*, [43]. These authors compared the plasma ivermectin concentration in two groups of 10 onchocerciasis patients, one receiving a standard dose of ivermectin with 750 ml of beer (corresponding to an average of 400 mg alcohol per kg body weight), and the other the same dose of ivermectin with 750 ml water. The dosages of ivermectin were done 1, 3 and 4 hours after treatment, and showed that the plasma concentrations were significantly higher in patients who took beer (66.3, 109.0, and 97.2 ng/ml at 1, 3 and 4 h, respectively) than in those who did not (44.0, 67.5, and 58.7 ng/ml, respectively) ($P < 0.01$ at each period).

It is clear that the doses of alcohol used by Shu *et al.*, [43] were fairly low and additional studies should be performed to evaluate the effect of higher quantities of alcohol on the bio-availability of ivermectin. In addition, whether this increased bio-availability is associated with an increased effect on the *Loa* microfilaraemia is difficult to ascertain. Comparisons of the effects of different doses of ivermectin on *Loa* have been done by Richard-Lenoble *et al.*, [44], who compared doses of 5, 10, 30, 50, 100, 150 and 200 μg/kg, Martin-Prével *et al.*, [22], who compared doses of 300 and 400 μg/kg, and Kamgno *et al.*, [19] with doses of 50 and 150 μg/kg. Richard-Lenoble *et al.*, [44] found that the decrease in *Loa* microfilaraemia at D2 and D7 were similar after doses of 50, 150 or 200 μg/kg. Martin-Prével *et al.*, [22] observed that the *Loa* microfilarial loads at D2 and D8–10 were lower, although not significantly, in those patients who received 400 μg/kg than in the ones treated at 300 μg/kg. In the last study, where examinations were done daily from D0 to D7, it was found that the microfilaraemia never differed significantly between the two groups of patients, although the counts in the group treated at the standard dose (150 μg/kg) were always lower than in the group treated with a low dose of 50 μg/kg [19].

From these results, one may assume that a standard dose of 150 μg/kg taken with alcohol would have similar effects that doses above 200 μg/kg, but it is difficult to assess whether the latter would have more effect on the *Loa* mf than the standard dose. However, it is interesting to keep in mind that the incidence of the "classical" side effects of ivermectin in onchocerciasis patients does not seem to be related to the plasma concentration of ivermectin [45] and/or the dose received [46,47]. In the two latter studies, the comparisons were done on doses of 150 and 800 μg/kg. In patients with lymphatic filariasis, the incidence of reactions is also similar after ivermectin

doses of 50, 100, and 200 μg/kg [48–52], and after doses of 150 or 400 μg/kg [53].

Lastly, besides the effect of the intake of classical alcoholic beverages (beer, palm wine, etc.), it would be most useful to perform studies to evaluate the effect of locally distilled beverages, which may contain various alcohols, and of chronic alcoholism, on the bio-availability of ivermectin.

Conclusions

The clinical and biological picture of post-ivermectin SAEs provides interesting information on the possible physiopathogenic processes leading to the condition, and thus to the best ways to prevent and/or to manage them.

The post-ivermectin Loa*-related SAEs are probably not due to a direct toxic effect of the drug*

As suggested by the title of the notice reporting the first case [28], one should first consider the possibility of a direct effect of ivermectin on the CNS. It is known that P-glycoproteins, which are drug-transporting proteins abundant in the endothelial cells of brain capillaries (part of the blood-brain barrier, BBB), limit the entry of ivermectin into the brain of normal animals, by transporting the drug from the inside of the cells to the outside. When treated with ivermectin, animals presenting a deficiency in P-glycoproteins develop a toxicosis related to high concentrations of the drug in the brain [54,55]. Although the phenomenon would certainly be rare [56], one may imagine that some individuals present a genetic deficiency in BBB P-glycoproteins. The spontaneous occurrence of genetic mutation of BBB P-glycoproteins has been demonstrated in mice [57,58], and is frequent in the collie dogs [59,60], which are the animals from whom cases of ivermectin toxicosis are the most frequently reported. In addition, a polymorphism of *MDR1* gene, associated with a variability of the expression of P-glycoprotein in the intestine, has been reported in humans [61–64]. However, there are a number of arguments against the direct implication of ivermectin in the SAEs reported above. First, the clinical picture is very different from the one reported in humans or animals developing an ivermectin toxicosis. In animals, besides the disorders of consciousness, they show ataxia, mydriasis, vomiting, drooling, muscle fasciculations, and apparent blindness, whereas in humans nausea/vomiting, salivation, tachycardia and hypotension are the most frequent signs of overdose [1]. None of the SAEs reported after a standard dose of ivermectin developed such a picture. Second, it would be surprising that a mutation in the *MDR1* gene would be limited to the geographic areas where the cases of SAEs have been reported.

What are the mechanisms of the post-ivermectin Loa*-related SAEs?*

It is clear that the main factor associated with the risk of post-ivermectin and post-DEC SAEs is the *Loa* microfilarial load. It is interesting to note that although the outcomes of these events occurring after ivermectin are usually less dramatic than those reported after DEC (i.e. in a simplified manner: 50% death, and 50% recovery with very serious sequelae), the threshold of microfilaraemia above which the encephalopathic process may occur is similar in both cases [30]. The processes which take place in the two types of SAE might thus well be the same. If this is the case, and as no pathological information is currently available for post-ivermectin SAEs, most interesting lessons can be drawn from the observations done on autopsy material of post-DEC fatal encephalopathies [65–68]. It is considered that these two drugs have different modes of action, but some similarities also exist between them. The fact that they provoke the passage of mf into urine and CSF (and, for *O. volvulus* mf, into the blood), and that there is a decrease in the eosinophil counts shortly after treatment, are such similar features.

Three main mechanisms could be proposed to explain the post-ivermectin SAEs, and as a matter of fact it is most likely that all of them occur simultaneously. The first one would be an obstructive process at the level of the cerebral microcirculation. The appearance of the retinal lesions described by Fobi *et al.*, [8], which have also been described after DEC, would suggest that a similar phenomenon also occurs in the brain. Whether this obstruction is related to the embolism of great numbers of mf paralysed by the drug, or to other processes is difficult to ascertain. The second mechanism would be that the mf, fleeing from the blood after treatment, go through the brain capillary endothelium and penetrate into the brain tissue. Even if the way in which the mf might do so cannot be explained, it is clear, from pathological observations done on a patient who died from a post-DEC SAE, that *Loa* mf are indeed able to penetrate into the brain tissue [66]. This would explain the diffuse EEG and scan abnormalities which have been observed in those cases which underwent such examinations. In this respect, one may also wonder whether the *Loa* mf do not cross the wall of the other capillaries to penetrate into all the connective tissues. This would explain the decrease in both the *Loa* microfilaraemia, and the eosinophil counts observed after ivermectin treatment. The third possible mechanism leading to the post-ivermectin SAEs would be the development of inflammatory processes at the cerebral level. It has been shown that in patients with onchocerciasis or lymphatic filariasis, the post-filaricide inflammatory reactions may be mediated by the release of products of *Wolbachia* endosymbionts [69,70]. Indeed, *Wolbachia* have also been found in *Loa loa*, but in such low quantity that

they are unlikely to contribute to the reactions developed by loiasis patients [71]. The inflammatory processes taking place in these patients would thus be probably mainly related to the filariae themselves. The release of *Loa* antigens, which has been demonstrated after ivermectin treatment [72], may play a role in the process.

How to reduce the pathological processes associated with the post-ivermectin Loa*-related SAEs?*

If the *Loa*-related post-ivermectin SAEs are partly related to inflammatory processes similar to the Mazzotti reaction, one might wonder what treatment would be the best to limit the phenomenon. The drugs which are the most often proposed for treatment of post-filaricide reactions are antihistamines and corticosteroids. Studies performed in Ghana have shown that antihistamines and indomethacin are without effect on the Mazzotti reaction occurring after DEC treatment of onchocerciasis patients [73,74]. Similarly, diphenhydramine was found to be ineffective in preventing reactions developing in patients infected with *Wuchereria bancrofti* who had been treated with DEC [75]. However, Carme *et al.*, [76] have observed that when antihistamines are co-administered with DEC, the severity (but not the incidence) of reactions is reduced; and Samé-Ekobo *et al.*, [77] reported that a daily antihistamine treatment by loratidine, during 10 days, brings about a decrease in the reactions which occurred in onchocerciasis patients treated with ivermectin.

As opposed to the antihistamines, corticosteroids seem most useful for dealing with the post-DEC reactions [27]. Anecdotal reports have suggested that these drugs or their related hormones may have beneficial effects [78]. It has been shown that starting a course of betamethasone or prednisone treatment 24–48 hours before the first dose of DEC in onchocerciasis patients reduces the severity of the Mazzotti reaction [79]. Awadzi *et al.*, [79,80] have also demonstrated that treatment with prednisone should be continued for at least 4 days after the first dose of DEC if one wants to avoid the rebound in the reaction after stopping the corticosteroids. However, co-administration of corticosteroids might also delay the destruction of the mf, and reduce the microfilaricidal activity. This is the reason why Stingl *et al.*, [74] evaluated whether starting dexamethasone treatment after the onset of the Mazzotti reaction would have an effect on the latter; these authors concluded that this was indeed so, but that the doses of corticosteroids had to be tapered rapidly.

If the post-ivermectin *Loa*-related SAEs are partly related to inflammatory processes similar to that associated with the Mazzotti reaction, our results on the changes in the eosinophil counts may suggest that the severity of the reactions could be reduced by administering corticosteroids within the two days following ivermectin treatment. At D3, the pathological processes are already in place, and the use of corticosteroids at that time might be too late to have any affect on the development of the reaction. Most of the cases of post-ivermectin SAEs have been managed using corticosteroids, and it seems that these drugs did not have any beneficial effect on the course of the condition. On the contrary, they may lead to the development of iatrogenic lethal complications. Additional studies should be performed to provide further information on the mechanisms of the post-ivermectin *Loa*-related SAEs. In this respect, one may consider the possibility of undertaking studies on animal models. The one which would probably reflect most closely what happens in humans would be drills (*Mandrillus leucophaeus*), mandrills (*Mandrillus sphinx*) or other monkeys experimentally infected with human *Loa loa*, and preferably splenectomised to obtain high microfilarial loads [81–85].

Competing interests

none declared

Authors' contribution

MB conceived, or participated in the studies on loiasis performed between 1993 and 2000 in the Laboratoire mixte Institut de Recherche pour le Développement-Centre Pasteur du Cameroun d'Epidémiologie et de santé publique; and he wrote the manuscript. JG and NGW supervised the field trial presented in [4] and [5], and NGW performed the hospital trial performed in 1993–1994 [7]. JPC conceived and supervised the latter trial. All authors read and approved the final manuscript.

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